

Amendments to the Specification

Please add the following paragraphs immediately after the title:

This is a division of Serial No. 09/568,143 filed May 10, 2000, now pending.

The prior application(s) set forth above are hereby incorporated by reference in their entirety.

Please replace the paragraph at page 9, lines 7-12 with the following amended paragraph:

The interaction of MBL with receptor/receptors on cells may be analysed by cytofluorimetry. 1) MBL at a concentration of 50 $\mu\text{g/ml}$ is incubated with ~~2x10⁵~~ 2x10⁵ cells. The binding is carried out in phosphate buffered salt solution (PBS) containing 1% FCS and 0.1% Na-azide. 2) For detection of cell-bound MBL, biotinylated anti-MBL antibody is applied; 3) followed by the addition of strepavidin-FITC and 4) analysis of the mixture by fluorimetry.

Please replace the paragraph beginning at line 20 of page 29 and ending at line 32 of page 30 with the following amended paragraph:

Human genomic DNA was isolated from 20 mL of blood drawn into EDTA. The EDTA/blood was mixed with 80 mL of cold (4°C) 10 mM Tris-HCl, 5 mM MgCl₂, 1% (v/v) Triton X-100, 0.32 mM sucrose, and left to incubate at 4°C for 30 min with occasional agitation. The sample was centrifuged at 1000xg for 30 min at 4°C, followed by discharge of the supernatant and a brief rinse of the nuclei-containing pellet in approximately 10 mL of 0.9% (w/v) of NaCl. The collected nuclei were resuspended in 6 mL of 75 mM NaCl, 24 mM EDTA [pH 8.0], and 460 μL of 10% (w/v) SDS and 1 mg pronase (cat. no. 165921, Boehringer-Mannheim, Mannheim, Germany) was added. After o/n incubation at RT with gentle agitation, 2 mL of a saturated (~ 6 M) NaCl solution was added. Following

vigorous agitation, the debris was removed by centrifugation at 1000xg. Isopropanol was added 1:1 to the supernatant and the vial was gently shaken until the DNA precipitate appeared. The thread-like precipitate was collected on a sealed Pasteur pipette, washed on the sealed tip in 70% (v/v) ethanol, air-dried and finally resuspended in 1 mL of TE-buffer. PCR amplification of the MBL gene was carried out with a sense-primer modified to contain an *Xho*I site: 5'-AGATTAACCTTCctcGAGTTTTCTCACACC-3' (SEQ ID NO:2). The anti-sense primer was modified to contain a *Bam*HI site: 5'-TAACggaTCCACTCCAATAATACATACAC-3' (SEQ ID NO:3) (restriction sites engineered into the sequences are underlined and modified basepairs compared to the natural gene sequence as published by Sastry et al. (1989) are shown as lower case letters in both primer sequences. Primers were produced by DNA technology, Aarhus, Denmark). The sense and anti-sense primers were located in the 5' and 3' UTRs respectively. Reaction conditions for the PCR consisted of 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 200 µM dTTP (cat. no. 1969064, Boehringer-Mannheim), reaction buffer at 1 x strength, 0.75 µL of enzyme (cat. no. 1732641, Expand High Fidelity™, Boehringer Mannheim, reaction buffer supplied with enzyme), genomic DNA (100 ng), and water to a final volume of 50 µL. The PCR thermal program performed 1 cycle at 96°C for 3 min, 10 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 5 min, followed by 20 cycle of 94°C for 15 s, 60°C for 30 s, and 68°C for 5 min with a time increment of 20 s per cycle, followed by a final elongation step at 72°C for 5 min (Omn 15 E™ thermal cycler, Hybaid, Ashford, UK). Cloning of the 6.2 kb PCR product into the pCR2 vector by use of the TA cloning kit™ (cat. no. K2000-01, Invitrogen, Leek, The Netherlands) was carried out according to the manufacturers' recommendations. The MBL gene was isolated from the pCR2 by digesting the vector with *Nde*I and *Bam*HI enzymes (cat. nos. 15441-025 & 15201-023, GibcoBRL, Paisley, UK) for 6 h at 37°C in an appropriate buffer. The insert DNA, i.e., the 5'-Not I-MBL-BamHI-3' fragment, was isolated from an 1% (w/v) agarose gel by

use of Pharmacia's Bandprep™ kit (cat. no. 27-9285-01, Pharmacia, Uppsala, Sweden). The pREP9 vector (cat. no. V009-50, Invitrogen) was digested as described for the MBL/pCR2 vector and the restriction product, i.e., the linearised vector, was likewise isolated from agarose to minimise contamination with undigested vector. In a final volume of 10 µL, 1 µL (~100 ng) of digested pREP9 vector, 4 µL (~400 ng) of 5'-NotI-MBL-BamHI-3', ligation buffer at 1x strength and 20 units of T4 ligase (cat. no. 202S, New England Biolabs, Beverly, MA, reaction buffer supplied with enzyme) was incubated o/n at 14°C. *E. coli* TOPF10 cells were made transformation competent by CaCl₂ permeabilising and transformed with the ligation product by heat-shock as described by Hanahan (1983). The bacteria were spread onto LB-agar plates supplemented with 50 µg ampicillin/mL, and colonies appeared after o/n incubation at 37°C.